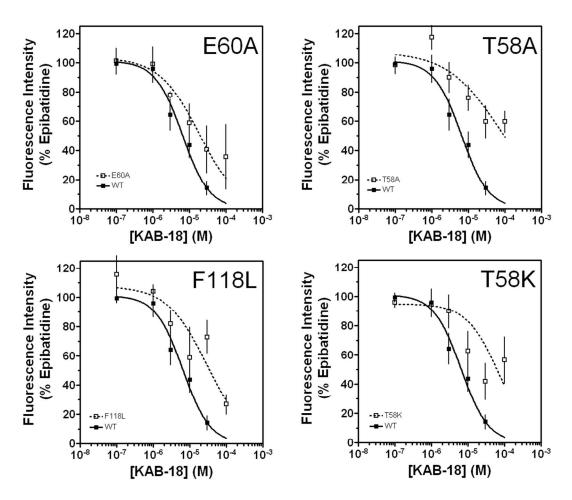


Supplemental Figure 1. Effects of KAB-18 on [3 H]Epibatidine to nAChRs. Competition binding experiments were performed on membrane homogenates from bovine adrenal medulla (BAM) (González-Cestari et al., 2009) or HEK tsA201 cells stably expressing human (H α 4 β 2) nAChRs using the indicated concentration of KAB-18. Data are expressed as a percentage of control specific binding. Values represent means \pm S.E.M. (n = 3-5).



Supplemental Figure 2. Concentration Response Effects of KAB-18 on WT and Mutant Transiently Transfected H α 4 β 2 nAChRs. Inhibition curves represented in Figure 3D of the main paper were separated for closer examination of the mutant and WT H α 4 β 2 nAChRs. Curves were fit to the exact same parameters as the compiled curves of Figure 3D.

Supplemental Methods.

[³H]Epibatidine Binding to Native and Recombinant nAChRs. Membrane preparations from bovine adrenal medullary (BAM) tissues or HEK tsA201 293 cells stably expressing Hα4β2 nAChRs were prepared, and binding assays were performed, as described previously by our laboratory (Free et al., 2003; González-Cestari et al., 2009). Briefly, membranes were incubated at room temperature for 60 min in buffer containing 1 nM (BAM) or 200 pM (Hα4β2) of [³H]epibatidine. Nonspecific binding was determined in the presence of 300 μM nicotine. Non-specific binding was typically 1 to 2% of total binding for recombinant nAChRs and 5 to 10% for native nAChRs.

Supplemental References

Free R.B., von Fischer N.D., Boyd R.T., and McKay D.B. (2003) Pharmacological characterization of recombinant bovine α3β4 neuronal nicotinic receptors stably expressed in HEK 293 cells. *Neurosci Lett* 343: 180-184.

Gonzalez-Cestari, T. F., Henderson, B. J., Pavlovicz, R. E., McKay, S. B., El-Hajj, R. A., Pulipaka, A. B., Orac, C. M., Reed, D. D., Boyd, R. T., Zhu, M. X., Li, C., Bergmeier, S. C., and McKay, D. B. (2009) Effect of novel negative allosteric modulators of neuronal nicotinic receptors on cells expressing native and recombinant nicotinic receptors: implications for drug discovery, *The Journal of pharmacology and experimental therapeutics* 328, 504-515.